# EVALUATION OF THE THERAPEUTIC EFFICACY OF AN INNOVATIVE HYDROGEL ENRICHED WITH EXTRACELLULAR VESICLES IN AN EXPERIMENTAL RAT MODEL OF COLITIS

**Bachelor's Degree Final Project**

Biochemistry and Molecular Biology

UNIVERSITAT ROVIRA i VIRGILI

**Laia Cabré Navarro**

Directed by Dra. Carolina Serena Perelló

Tarragona, June 2025

## CONFIDENTIAL PROJECT

This project has been developed from the experience and data obtained during my curricular internship carried out in the Inflammatory Bowel Diseases Research Group (IBODI), from Institut d'Investigació Sanitària Pere Virgili (IISPV), at Joan XXIII University Hospital in Tarragona, under the supervision of Dra. Carolina Serena Perelló.

The content of this project is **confidential**, and it is subject to a non-disclosure agreement between URV, IISPV, and the author.

# Table of Contents

## Abbreviations

| | |
|---|---|
| **AdMSC** | Adipose-derived Mesenchymal Stem Cell |
| **BCA** | Bicinchoninic Acid |
| **BSA** | Bovine Serum Albumin |
| **CCM** | Concentrated Conditioned Medium |
| **CM** | Conditioned Medium |
| **$CaCl_2$** | Calcium Chloride |
| **Cryo-TEM** | Cryogenic Transmission Electron Microscopy |
| **DAHP** | 1,6-Diaminohexane phosphate |
| **DAI** | Disease Activity Index |
| **DG** | Dulbecco's Modified Eagle Medium: High Glucose/Ham's F12 Medium |
| **DSS** | Dextran Sulphate Sodium |
| **EV** | Extracellular Vesicle |
| **FBS** | Fetal Bovine Serum |
| **HAT** | Healthy Adipose Tissue |
| **IBD** | Inflammatory Bowel Disease |
| **IFN-γ** | Interferon gamma |
| **IL** | Interleukin |
| **M1/M2** | M1/M2 Macrophages |
| **MSC** | Mesenchymal Stem Cell |
| **NTA** | Nanoparticle Tracking Analysis |
| **PBS** | Phosphate-Buffered Saline |
| **PEG** | Polyethylene Glycol |
| **SAT** | Subcutaneous Adipose Tissue |
| **SEC** | Size Exclusion Chromatography |
| **SVF** | Stromal Vascular Fraction |
| **TGF-β** | Transforming Growth Factor beta |
| **TLR** | Toll-like Receptor |
| **TNBS** | 2,4,6-Trinitrobenzenesulfonic Acid |
| **TNF-α** | Tumor Necrosis Factor alpha |
| **Th1/Th17** | T helper type 1 / T helper type 17 cells |
| **Treg** | Regulatory T cells |

## Abstract

This study explores a novel therapeutic approach based on extracellular vesicles (EVs) derived from adipose-derived mesenchymal stem cells, delivered locally using a biocompatible hydrogel system. EVs were successfully isolated, characterized, and incorporated into the delivery platform, which enabled sustained in vitro release and preserved vesicle integrity. In a preclinical model of colitis, treatment with the EV-enriched system led to reduced disease severity, improved clinical outcomes, and preservation of tissue structure. These findings support the potential of localized, cell-free therapies to enhance EVs stability, bioavailability, and therapeutic efficacy in inflammatory bowel disease.

# 1. Introduction

## 1.1. Crohn's Disease

### 1.1.1. Definition

Inflammatory bowel disease (IBD) encompasses a group of chronic, relapsing inflammatory disorders that primarily affect the gastrointestinal tract. The two main subtypes are Crohn's disease and ulcerative colitis [1]. While both conditions share common features, they differ significantly in their distribution and pathological characteristics. Ulcerative colitis is restricted to the colon and involves continuous inflammation of the mucosal layer**,** typically starting at the rectum and extending proximally. In contrast, Crohn's disease can affect any part of the gastrointestinal tract**,** from the mouth to the anus**,** and is characterized by transmural inflammation, meaning it affects the entire thickness of the intestinal wall [1,2].

Crohn's disease is characterized by an inflammatory immune response that alternates between periods of activity and remission, associated with poor quality of life. Its ethology is not completely understood, although it is believed that genetic background and environmental factors play a role in altering the intestinal microbiota that leads to a dysbiosis which contributes to the pathophysiology of the disease [1,2]. The symptoms experienced in Crohn's disease can be quite diverse and may include abdominal pain, diarrhoea, stomach discomfort, weight loss, nausea, vomiting, and in instances, fever or chills [3].

### 1.1.2. Epidemiology and Etiopathogenesis

The incidence of Crohn's disease varies based on the geographical region but is increasing worldwide, with the highest rates observed in developed countries like Western Europe and North America, where it can exceed 3 to 20 cases per 100,000 [3–5]. In recent years, there has been an increase in cases in regions like Asia, South America, and the Middle East, this suggests that environmental and lifestyle changes are involved in the disease's development [3].

Crohn's disease primarily affects individuals between the ages of 15 and 35, although it can affect people of all ages, it typically shows two incidence peaks at 20-40 years and 50-60 years [2,6]. The disease affects men and women equally. The increasing incidence

is attributed to improvements in diagnostic technology and changes in environmental and dietary factors, highlights its growing significance as a public health concern [6].

Crohn's disease is a complex, multifactorial condition resulting from the interaction of genetic, immune, microbial, and environmental factors [1]. Specific genetic mutations, especially in the NOD2/CARD15 gene, are strongly associated to an increased risk of developing Crohn's disease, as these mutations impair the ability to recognize pathogens in the gut, triggering an inflammatory response [7]. Other genes, such as ATG16L1, IRGM, IL23R, and TLR, also contribute to genetic susceptibility, affecting immune response and protection against infections [7,8]. This results in an unusual immune reaction, where the body's immune system attacks the gut microbiota and its own tissues, resulting in ongoing inflammation [9]. This dysregulation involves both innate and adaptive immune cells, with elevated levels of inflammatory cytokines like IL-6 and IL-12 in inflamed tissue [9,10].

The gut microbiome plays a critical role in the pathogenesis of the disease, comprising bacteria and microorganisms that regulate digestion and modulate immune responses. In Crohn's disease patients, the microbiome is often disrupted, leading to a decrease in beneficial bacteria and an overgrowth of harmful microorganisms, a condition known as dysbiosis [10]. This imbalance contributes to immune system dysfunction, promoting chronic inflammation [3]. In particular, the altered microbiota in Crohn's disease patients is characterized by lower diversity and a rise in pro-inflammatory species like adherent-invasive *Escherichia coli*, which worsens intestinal barrier dysfunction [11]. This imbalance in the microbiome can also lead to an atypical immune reaction towards commensal bacteria, perpetuating the inflammatory cycle and contributing to the chronic nature of the disease [10].

Environmental factors are also crucial in the development of the pathology, influencing its onset and progression. Urbanization, pollution, dietary habits, and reduced microbial exposure are all linked to a higher risk of developing Crohn's disease [4]. Early-life factors, such as prenatal exposure to antibiotics, passive smoking, and infections, increase susceptibility. Smoking, in particular, is one of the most well-established risk factors, nearly doubling the likelihood of developing the pathology and contributing to more severe disease progression. Alternatively, exposure to animals appears to lower the risk of IBD, possibly by enhancing microbial diversity and promoting a balanced immune response [12]. These environmental influences, in combination with genetic

predisposition, underscore the rising incidence and the importance of lifestyle factors in disease development.

Understanding these multifaceted risk factors provides opportunities for developing innovative diagnostic tools and therapeutic strategies. By targeting the gut microbiome and addressing immune system dysfunction, researchers can potentially improve the management and treatment of Crohn's disease, offering hope for more effective interventions in the future [12].

### 1.1.3. Pathophysiology

The pathophysiology involves an interplay of innate and adaptive immune systems, with overactivation of Toll-like receptors (TLR-2 and TLR-4) on dendritic cells and macrophages, resulting in excessive production of pro-inflammatory cytokines (IL-6, IL-12, IL-23) and recruitment of Th1 and Th17 lymphocytes. This is exacerbated by a lack of anti-inflammatory mechanisms, including reduced regulatory T cells (Treg) and IL-10 production [13,14]. In the inflamed mucosa of Crohn's disease patients is observed an aberrant immune response, marked by persistent activation of effector T lymphocytes and disruption of regulatory T cell (Treg) homeostasis. Additionally, an increase in activated antigen-presenting cells sustains the inflammatory cycle through high TNF-α, IL-6, and IL-23 production, contributing to ongoing tissue damage [15].

The chronic inflammatory state promotes the expansion of mesenteric adipose tissue, known as "creeping fat", which is a hallmark of patients with Crohn's disease [16]. The creeping fat can cover up to half of the intestine and serves as a reservoir for bacteria and memory T cells, perpetuating inflammation and contributing to disease recurrence. Loss of intestinal immune barrier integrity facilitates bacterial translocation to mesenteric fat, where adipocytes and resident monocytes detect microbial components through pattern recognition receptors. This cause adipocyte hypertrophy and release pro-inflammatory mediators such as TNF-α, leptin and free fatty acids, promoting fibrosis and tissue damage [16].

Following bacterial translocation, monocytes in the mesenteric fat differentiate into macrophages, mainly adopting a pro-inflammatory M1 phenotype due to the influence of lipopolysaccharides and cytokines, which results in enhanced antigen presentation, phagocytosis, and the release of inflammatory cytokines [16,17]. At the same time, $CD4^+$ T cells differentiate into Th1 and Th17, which play a role in inflammation by producing

IFN-γ, IL-12, IL-17, and IL-22 [17]. Although regulatory T cells (Treg) are present, their ability to supress the immune response driven by IL-10 and TGF-β, is insufficient to balance the inflammatory response [16,17]. These effects are summarized in **Figure 1**.

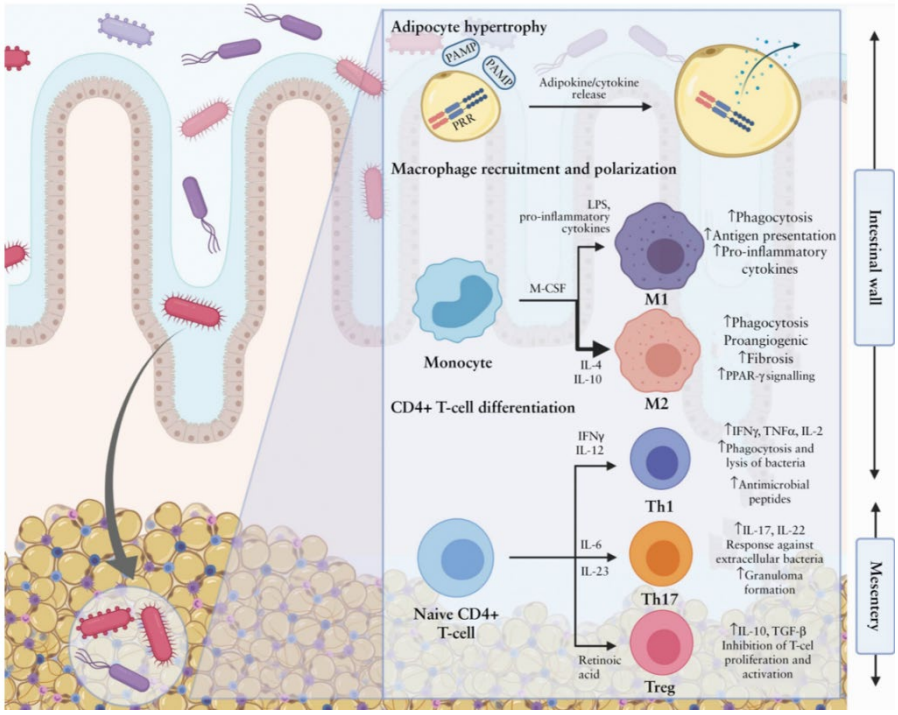


***Figure 1.*** *Effects of bacterial translocation on creeping fat and immune cell activation. (From [16]).*

Creeping fat can lead to various complications, including fibrostenosis, which affects about one-third of Crohn's disease patients. It causes strictures (narrowing of intestinal sections) and intestinal fibrosis, potentially requiring surgical intervention [16,18]. Studies have shown that patients with creeping fat have a higher risk of developing bowel damage and needing surgery within two years of diagnosis [18].

This complex interplay creates a vicious cycle of immune dysregulation and tissue remodelling, ultimately leading to transmural inflammation, fibrosis, and complications such as strictures and fistulas characteristic of the pathology [14].

### 1.1.4. Clinical Management

The main goals of Crohn's disease treatment are to induce and maintain clinical remission, prevent disease progression, and optimize nutritional status [19].

Pharmacological treatment is crucial to management. Corticosteroids are often used for acute flare-ups, while aminosalicylates are typically prescribed for cases involving mild

colonic inflammation [3]. For long-term management, immunomodulators like thiopurines and methotrexate are commonly chosen. In moderate to severe cases, biologic therapies are essential for treatment. These include anti-TNFα agents such as infliximab and adalimumab, anti-integrin drugs like vedolizumab, and IL-12/23 inhibitors, for example, ustekinumab [19].

Monitoring disease activity is key for adjusting treatment. Regular clinical evaluations, biomarkers like fecal calprotectin; endoscopic assessments, and imaging techniques help to assess response to therapy [3].

Even with medical treatment, a considerable proportion of patients (40-60%) may require a surgery within 10 years of their diagnosis [19]. Surgical options include stricturoplasty, resection with anastomosis, or diverting ostomy, depending on complications [20]. Despite therapeutic advances, approximately 50% of patients still undergo surgical intervention, and up to 80% of them experience recurrence at the site of surgery, often requiring a second procedure [18–20]. Since no cure currently exists for this disease, it is essential to discover new treatments to avoid these invasive methods. Extracellular vesicles present a promising therapeutic option.

## 1.2. Therapeutic Potential of Extracellular Vesicles derived from Adipose-derived Mesenchymal Stem Cells

### 1.2.1 Extracellular Vesicles

Extracellular vesicles (EVs) are structures enclosed by lipid bilayers that are secreted by cells into the extracellular space, and they represent a mechanism used for intercellular communication [21]. This process is based on their ability to transport biomolecules such as proteins, lipids, nucleic acids, and sugars, allowing information to be transmitted between cells [22]. This function makes them highly relevant in the regulation of various biological processes like the immune response, inflammation, or tissue regeneration [22]. These vesicles are present in a wide range of organisms, from simple to complex, which means this mechanism has been conserved throughout evolution [23].

EVs are commonly classified into three main types based on their origin, biogenesis, and molecular content: exosomes, microvesicles and apoptotic bodies [21]. Exosomes are formed within multivesicular bodies and released when these bodies fuse with the plasma membrane [24,25]. The ESCRT (Endosomal Sorting Complex Required for Transport)

complex plays a key role in sorting and packaging cargo for exosome formation [24]. Microvesicles, in contrast, are generated by direct budding from the plasma membrane, a process that requires the clustering of lipids and proteins into specific domains for subsequent membrane scission [25]. Apoptotic bodies are formed during programmed cell death (apoptosis), when the cell fragments its membrane, releases vesicles containing cellular components like proteins and nucleic acids [26]. The molecular composition and size of EVs is different depending on their cellular origin and the physiological or pathological context but are generally within the nanoscale range [23].

### 1.2.2. Extracellular Vesicles derived from Adipose-derived Mesenchymal Stem Cells

Adipose-derived mesenchymal stem cells (AdMSCs) are a type of multipotent stem cell that can be easily obtained from adipose tissue through procedures that are minimally invasive, such as liposuction [27]. These cells exhibit a strong ability of adhesion to culture surfaces, proliferation efficiently *in vitro*, and differentiating into various mesoderm-derived cell types, including adipogenic, osteogenic, chondrogenic, myogenic, angiogenic, cardiomyogenic, tenogenic, and periodontogenic lineages [28]. These characteristics make them particularly attractive in the field of regenerative medicine.

AdMSCs have shown effectiveness in the repair of tissue damage caused by radiotherapy, and they have been used in the treatment of severe hematological and immunological diseases, suggesting a significant immunomodulatory function [28,29].

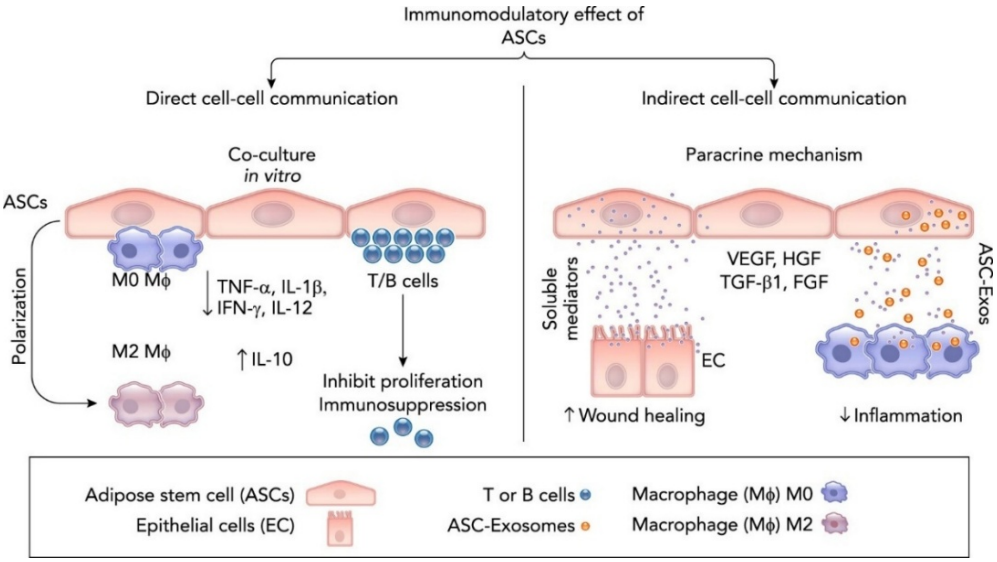


***Figure 2.*** *Immunomodulatory mechanisms of adipose-derived stem cells through direct and indirect cell communication. (From [30]).*

As illustrated in **Figure 2**, AdMSCs show immunomodulatory effects through both direct and indirect communication with immune and epithelial cells. In direct cell-cell interactions (left side of the figure), AdMSCs can polarize naïve M0 macrophages toward an anti-inflammatory M2 phenotype, increasing IL-10 production and reducing pro-inflammatory cytokines such as TNF-α, IL-1β, IFN-γ, and IL-12 [28,30].

Furthermore, AdMSCs inhibit the proliferation of T and B lymphocytes, contributing to immunosuppression. On the other hand, indirect mechanisms (right side of the figure) involve paracrine signalling through the secretion of soluble mediators and EVs, including ADMSC-derived exosomes [31]. These bioactive components promote wound healing and reduce inflammation by delivering molecules such as VEGF, HGF, TGF-β1, and FGF to target cells [30]. These complex actions highlight the therapeutic relevance of ADMSC-derived EVs as a promising, cell-free approach for regenerative and immunomodulatory treatments.

### 1.2.3. Therapeutic Applications in Inflammatory Bowel Diseases

While AdMSC-based therapies have shown promising immunomodulatory and regenerative effects in preclinical and early clinical studies for inflammatory bowel disease [30,32], their clinical application remains limited due to safety concerns, poor post-administration cell viability, immune rejection risks, and high production costs [33].

To overcome these limitations, EVs derived from AdMSCs have emerged as a promising alternative. These EVs exhibit similar anti-inflammatory and immunomodulatory properties to their parental cells, such as the reduction of pro-inflammatory cytokines like TNF-α and IFN-γ. Additionally, EVs have been shown to decrease the expression of IL-12, a cytokine that plays a critical role in IBD pathogenesis by promoting the differentiation of naïve T cells into Th1 effector cells, which are key drivers of chronic inflammation [34].

Compared to AdMSCs, EVs derived from AdMSCs offer several advantages, including improved safety, enhanced stability, and reduced immunogenicity, making them a potentially more viable therapeutic option [33].

Recent preclinical studies using induced colitis models in mice have demonstrated that AdMSC-derived EVs significantly reduce clinical disease activity indices, restore colonic

architecture, and improve epithelial barrier integrity [35,36]. These therapeutic effects are closely associated with a marked downregulation of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, IFN-γ) and an upregulation of the anti-inflammatory cytokine IL-10. Furthermore, AdMSC-EVs appear to reprogram macrophage polarization from a pro-inflammatory M1 phenotype towards an anti-inflammatory M2 profile, contributing to local immune resolution.

In more specific IBD contexts, such as Crohn's disease, AdMSC-EVs derived from creeping fat, a hypertrophied adipose tissue characteristic of Crohn's pathology, have been shown to improve lymphatic drainage and reduce inflammation in the mesentery in IL-10 knockout mice, offering a novel therapeutic angle targeting the gut-lymphatic interface [37].

Although clinical data are still emerging, early-phase trials indicate that AdMSC-EVs are well tolerated when administered locally or systemically, and preliminary outcomes suggest efficacy in reducing inflammation and promoting mucosal healing [38]. Based on these results, EVs derived from AdMSCs offer a promising alternative to traditional stem cell therapies. They show similar therapeutic effects but with added benefits, such as being easier to store, safer to use, and more practical to produce on a large scale. However, further clinical studies are warranted to fully validate their use as a next-generation therapy for Crohn's disease and other forms of IBD.

## 1.3. Encapsulation Strategies of Extracellular Vesicles using Biomaterials

Even with their encouraging potential, EVs face multiple challenges when applied *in vivo*, including low stability, rapid degradation, and uncontrolled biodistribution [39]. These limitations hinder their clinical application and highlight the necessity for strategies that can protect EVs and improve their delivery efficiency.

The encapsulation of EVs using biomaterials has emerged as a promising strategy to overcome current limitations. Hydrogels are receiving increasing attention because they provide a hydrated three-dimensional environment that closely mimics an extracellular matrix [40]. A wide range of biomaterials are being investigated for EVs encapsulation, including natural polymers such as collagen, gelatine, chitosan, hyaluronic acid and alginate, as well as synthetic materials like polyethylene glycol, polylactic-co-glycolic acid, and polyhydroxy ethyl methacrylate. In some cases, combinations of both natural and synthetic biomaterials are also being explored [40,41].

# 2. Hypothesis and Objectives

## 2.1. Hypothesis

The encapsulation of EVs derived from AdMSCs in an xxxxx-based hydrogel will significantly enhance their therapeutic efficacy in a TNBS-induced rat model of colitis. This delivery strategy is expected to improve the stability, bioavailability, and controlled release of EVs, thereby promoting tissue repair, reducing xxxxx compared to the administration of non-encapsulated EVs.

## 2.2. Objectives

**General objective:** To design, develop, and evaluate an xxxxx-based hydrogel system for the targeted and efficient delivery of AdMSC-derived EVs in experimental colitis.

**Specific objectives:**

1. To isolate and characterize EVs derived from AdMSCs.
2. To fabricate an xxxxx-based biomaterial suitable for EVs encapsulation.
3. To analyse the release kinetics of EVs from the hydrogel using nanoparticle tracking analysis (NTA) and MicroBCA.
4. To evaluate the therapeutic efficacy of encapsulated EVs in a TNBS-induced colitis rat model.

## 3. Materials and Methods

EVs were derived from adipose-derived mesenchymal stem cells isolated from healthy donors. Cells were expanded under standard culture conditions, and EV-enriched conditioned medium was collected. EVs were purified using size exclusion chromatography and characterized by protein quantification assays, nanoparticle tracking analysis, flow cytometry (positive for relevant surface markers), and cryo-transmission electron microscopy.

A biocompatible matrix was developed and optimized based on its physicochemical properties. EVs were incorporated into this matrix, and release kinetics were evaluated *in vitro* over several days using a transwell-based system.

To assess therapeutic potential, the EV-containing formulation was applied locally in a preclinical model. Disease progression was monitored through imaging techniques, pathological tissue assessment, and clinical activity indices.

## 4. Results

EVs demonstrated the expected size distribution and surface marker profile, consistent with previously reported characteristics. The formulated delivery matrix allowed for efficient encapsulation and exhibited a sustained *in vitro* release of EVs over time.

*In vivo* application of the EV-enriched formulation resulted in measurable improvement in clinical scores and a visible reduction of both internal and external signs of xxxxx, when compared to untreated controls.

## 5. Discussion

This study presents the development and evaluation of a novel cell-free therapeutic platform based on the encapsulation of EVs into a biocompatible matrix designed for local delivery. Our results indicate that this formulation supports sustained release *in vitro* and enhances therapeutic outcomes *in vivo*, as shown by improved clinical and structural indicators of xxxxx.

EVs were successfully isolated, characterized using multiple analytical techniques, and incorporated into the delivery system at defined concentrations. Local administration of this EV-based platform, which may reduce the need for repeated systemic dosing while improving local bioavailability.

These preliminary findings highlight the potential of matrix-assisted EVs delivery as a promising therapeutic strategy for inflammatory bowel diseases and warrant further investigation to validate efficacy, understand mechanisms of action, and consider pathways toward clinical implementation.

## 6. Conclusions

This pilot study demonstrates the therapeutic potential of an innovative biocompatible delivery system enriched with EVs derived from human mesenchymal stem cells. The system enabled a sustained *in vitro* release of EVs over several days while preserving vesicle integrity.

*In vivo* application showed clear therapeutic benefits, including improvements in clinical scores and macroscopic indicators of xxxxx when compared to controls. These findings support the initial hypothesis that encapsulating EVs within a delivery matrix enhances their stability, bioavailability, and local therapeutic impact. All key objectives of the study were successfully met, including EVs isolation and characterization, formulation development, release profiling, and preclinical evaluation.

Future studies should address current limitations by expanding the sample size, including both sexes, and incorporating molecular and histological analyses to better understand mechanisms of action. Long-term efficacy and scalability will also be critical aspects for potential clinical translation.

## 7. Acknowledgements

*Primer de tot, m'agradaria agrair a la Dra. Carolina Serena, que com a IP del grup IBODI ha confiat en mi des del primer moment per realitzar les practiques i el TFG amb ells. M'ha acollit com una més i m'ha fet sentir part del grup des del primer dia. També li agraeixo que hagi supervisat i tutoritzat aquest treball amb tanta dedicació, ajudant-me perquè tot sortís perfecte.*

*En segon lloc, moltes gràcies a totes les companyes i al company del grup. A la Dra. Laura Clua per guiar-me, ensenyar-me i confiar en mi; a l'Albert Boronat per ajudar-me sempre que ho he necessitat. Però especialment vull agrair a la Dra. Diandra Monfort: gràcies per deixar-me aprendre al teu costat, per tota la confiança i per fer que el dia a dia, tan dins com fora del laboratori, fos sempre una experiència positiva. Ets una part molt important d'aquest treball i d'aquesta etapa.*

*També voldria agrair l'ajuda i participació del grup IBD de l'Institut de Recerca Germans Trias i Pujol (IGTP), dirigit pel Dr. Josep Manyé, en tot el desenvolupament de l'avaluació in vivo. En especial, al Roger Suau, que m'ha ajudat molt en l'obtenció i interpretació de les dades i resultats.*

*Per últim, a la meua família, que sou una part de mi: gràcies per escoltar les actualitzacions de les cel·luletes (com dieu vatros) amb paciència i un somriure, encara que no enteníeu del tot el que vos explicava. Gràcies per animar-me sempre a tirar endavant, per aguantar i entendre els meus dies de cansament, i celebrar amb mi cada pas, per menut que fos. Vos estimo molt.*

# 8. References


[1] Dotson JL, Boyle B. Crohn Disease. Pediatric Gastrointestinal and Liver Disease, Sixth Edition 2024:461-473.e8. https://doi.org/10.1016/B978-0-323-67293-1.00042-6.

[2] Ballester Ferré MP, Boscá-Watts MM, Mínguez Pérez M. Crohn's disease. Med Clin (Barc) 2018;151:26–33. https://doi.org/10.1016/J.MEDCLI.2017.10.036.

[3] Feuerstein JD, Cheifetz AS. Crohn Disease: Epidemiology, Diagnosis, and Management. Mayo Clin Proc 2017;92:1088–103. https://doi.org/10.1016/J.MAYOCP.2017.04.010.

[4] Gajendran M, Loganathan P, Catinella AP, Hashash JG. A comprehensive review and update on Crohn's disease. Dis Mon 2018;64:20–57. https://doi.org/10.1016/J.DISAMONTH.2017.07.001.

[5] Caron B, Honap S, Peyrin-Biroulet L. Epidemiology of Inflammatory Bowel Disease across the Ages in the Era of Advanced Therapies. J Crohns Colitis 2024;18:ii3–15. https://doi.org/10.1093/ECCO-JCC/JJAE082.

[6] Agrawal M, Jess T. Implications of the changing epidemiology of inflammatory bowel disease in a changing world. United European Gastroenterol J 2022;10:1113–20. https://doi.org/10.1002/UEG2.12317.

[7] Ashton JJ, Seaby EG, Beattie RM, Ennis S. NOD2 in Crohn's Disease-Unfinished Business. J Crohns Colitis 2023;17:450–8. https://doi.org/10.1093/ECCO-JCC/JJAC124.

[8] Liu JZ, Anderson CA. Genetic studies of Crohn's disease: past, present and future. Best Pract Res Clin Gastroenterol 2014;28:373–86. https://doi.org/10.1016/J.BPG.2014.04.009.

[9] Xu XR, Liu CQ, Feng BS, Liu ZJ. Dysregulation of mucosal immune response in pathogenesis of inflammatory bowel disease. World Journal of Gastroenterology : WJG 2014;20:3255. https://doi.org/10.3748/WJG.V20.I12.3255.

[10] Caparrós E, Wiest R, Scharl M, Rogler G, Gutiérrez Casbas A, Yilmaz B, et al. Dysbiotic microbiota interactions in Crohn's disease. Gut Microbes 2021;13. https://doi.org/10.1080/19490976.2021.1949096.

[11] Foppa C, Rizkala T, Repici A, Hassan C, Spinelli A. Microbiota and IBD: Current knowledge and future perspectives. Dig Liver Dis 2024;56:911–22. https://doi.org/10.1016/J.DLD.2023.11.015.

[12] Singh N, Bernstein CN. Environmental risk factors for inflammatory bowel disease. United European Gastroenterol J 2022;10:1047–53. https://doi.org/10.1002/UEG2.12319.

[13] Petagna L, Antonelli A, Ganini C, Bellato V, Campanelli M, Divizia A, et al. Pathophysiology of Crohn's disease inflammation and recurrence. Biol Direct 2020;15. https://doi.org/10.1186/S13062-020-00280-5.

[14] Wallace KL, Zheng LB, Kanazawa Y, Shih DQ. Immunopathology of inflammatory bowel disease. World J Gastroenterol 2014;20:6–21. https://doi.org/10.3748/WJG.V20.I1.6.

[15] Xu XR, Liu CQ, Feng BS, Liu ZJ. Dysregulation of mucosal immune response in pathogenesis of inflammatory bowel disease. World Journal of Gastroenterology : WJG 2014;20:3255. https://doi.org/10.3748/WJG.V20.I12.3255.

[16] Suau R, Pardina E, Domènech E, Lorén V, Manyé J. The Complex Relationship Between Microbiota, Immune Response and Creeping Fat in Crohn's Disease. J Crohns Colitis 2022;16:472–89. https://doi.org/10.1093/ECCO-JCC/JJAB159.

[17] Panda SK, Colonna M. Innate Lymphoid Cells in Mucosal Immunity. Front Immunol 2019;10. https://doi.org/10.3389/FIMMU.2019.00861.

[18] Aggeletopoulou I, Tsounis EP, Mouzaki A, Triantos C. Creeping Fat in Crohn's Disease-Surgical, Histological, and Radiological Approaches. J Pers Med 2023;13. https://doi.org/10.3390/JPM13071029.

[19] Cushing K, Higgins PDR. Management of Crohn Disease: A Review. JAMA 2021;325:69–80. https://doi.org/10.1001/JAMA.2020.18936.

[20] Colombo F, Frontali A, Baldi C, Cigognini M, Lamperti G, Manzo CA, et al. Repeated surgery for recurrent Crohn's disease: does the outcome keep worsening operation after operation? A comparative study of 1224 consecutive procedures. Updates Surg 2021;74:73. https://doi.org/10.1007/S13304-021-01187-0.

[21] Doyle LM, Wang MZ. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells 2019;8:727. https://doi.org/10.3390/CELLS8070727.

[22] Yáñez-Mó M, Siljander PRM, Andreu Z, Zavec AB, Borràs FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles 2015;4:10.3402/jev.v4.27066. https://doi.org/10.3402/JEV.V4.27066.

[23] Zaborowski MP, Balaj L, Breakefield XO, Lai CP. Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study. Bioscience 2015;65:783. https://doi.org/10.1093/BIOSCI/BIV084.

[24] Van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nature Reviews Molecular Cell Biology 2018 19:4 2018;19:213–28. https://doi.org/10.1038/nrm.2017.125.

[25] Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 2014;30:255–89. https://doi.org/10.1146/ANNUREV-CELLBIO-101512-122326/CITE/REFWORKS.

[26] Ge Y, Ye T, Fu S, Jiang X, Song H, Liu B, et al. Research progress of extracellular vesicles as biomarkers in immunotherapy for non-small cell lung cancer. Front Immunol 2023;14:1114041. https://doi.org/10.3389/FIMMU.2023.1114041/XML/NLM.

[27] Bassi G, Pacelli L, Carusone R, Zanoncello J, Krampera M. Adipose-derived stromal cells (ASCs). Transfusion and Apheresis Science 2012;47:193–8. https://doi.org/10.1016/j.transci.2012.06.004.

[28] Si Z, Wang X, Sun C, Kang Y, Xu J, Wang X, et al. Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies. Biomedicine & Pharmacotherapy 2019;114:108765. https://doi.org/10.1016/J.BIOPHA.2019.108765.

[29] Kuca-Warnawin E, Plebańczyk M, Ciechomska M, Olesińska M, Szczęsny P, Kontny E. Impact of Adipose-Derived Mesenchymal Stem Cells (ASCs) of Rheumatic Disease Patients on T Helper Cell Differentiation. Int J Mol Sci 2022;23:5317. https://doi.org/10.3390/IJMS23105317/S1.

[30] Al-Ghadban S, Bunnell BA. Adipose tissue-derived stem cells: Immunomodulatory effects and therapeutic potential. Physiology 2020;35:125–33. https://doi.org/10.1152/PHYSIOL.00021.2019/ASSET/IMAGES/LARGE/PHY001200498001.JPEG.

[31] Műzes G, Sipos F. Mesenchymal Stem Cell-Derived Secretome: A Potential Therapeutic Option for Autoimmune and Immune-Mediated Inflammatory Diseases. Cells 2022, Vol 11, Page 2300 2022;11:2300. https://doi.org/10.3390/CELLS11152300.

[32] Saadh MJ, Mikhailova M V., Rasoolzadegan S, Falaki M, Akhavanfar R, Gonzáles JLA, et al. Therapeutic potential of mesenchymal stem/stromal cells (MSCs)-based cell therapy for inflammatory bowel diseases (IBD) therapy. European Journal of Medical Research 2023 28:1 2023;28:1–24. https://doi.org/10.1186/S40001-023-01008-7.

[33] Zhou Y, Yamamoto Y, Xiao Z, Ochiya T. The immunomodulatory functions of mesenchymal stromal/stem cells mediated via paracrine activity. J Clin Med 2019;8. https://doi.org/10.3390/JCM8071025,.

[34] Dadgar N, Altemus J, Li Y, Lightner AL. Effect of Crohn's disease mesenteric mesenchymal stem cells and their extracellular vesicles on T-cell immunosuppressive capacity. J Cell Mol Med 2022;26:4924–39. https://doi.org/10.1111/JCMM.17483,.

[35] Li Y, Altemus J, Lightner AL. Mesenchymal stem cells and acellular products attenuate murine induced colitis. Stem Cell Res Ther 2020;11:1–11. https://doi.org/10.1186/S13287-020-02025-7/FIGURES/6.

[36] Lee JH, Lötvall J, Cho BS. The Anti-Inflammatory Effects of Adipose Tissue Mesenchymal Stem Cell Exosomes in a Mouse Model of Inflammatory Bowel Disease. Int J Mol Sci 2023;24:16877. https://doi.org/10.3390/IJMS242316877/S1.

[37] Shu W, Wang Y, Chen M, Zhu X, Wang F, Chen C, et al. Extracellular vesicles derived from creeping fat stem cells promote lymphatic function and restrain inflammation of Crohn's disease. Clin Transl Med 2024;14. https://doi.org/10.1002/CTM2.70086.

[38] Hadizadeh A, Akbari-asbagh R, Heirani-tabasi A, Soleimani M, Gorovanchi P, Ebrahimi Daryani N, et al. Localized Administration of Mesenchymal Stem Cell-Derived Exosomes for the Treatment of Refractory Perianal Fistula in Crohn's Disease Patients: A Phase II Clinical Trial. Dis Colon Rectum 2024;67. https://doi.org/10.1097/DCR.0000000000003502,.

[39] van Niel G, Carter DRF, Clayton A, Lambert DW, Raposo G, Vader P. Challenges and directions in studying cell–cell communication by extracellular vesicles. Nature Reviews Molecular Cell Biology 2022 23:5 2022;23:369–82. https://doi.org/10.1038/s41580-022-00460-3.

[40] Riau AK, Ong HS, Yam GHF, Mehta JS. Sustained delivery system for stem cell-derived exosomes. Front Pharmacol 2019;10:493943. https://doi.org/10.3389/FPHAR.2019.01368/BIBTEX.

[41] Peppas NA, Hilt JZ, Khademhosseini A, Langer R. Hydrogels in Biology and Medicine: From Molecular Principles to Bionanotechnology. Advanced Materials 2006;18:1345–60. https://doi.org/10.1002/ADMA.200501612.